

The Study of Some Bioaerosols (Bacterial) Pollution in Air of Wastewater Treatment Plant in Cold Months

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Abstract

Numerous pathogenic and non-pathogenic microorganisms are present in the wastewater. These bacteria have the potential to transform into bioaerosols throughout the treatment process, endangering the health of plant employees. The results of the study showed the highest mean number of bacteria (mean 150.0 CFU/m³) in November was observed in the aeration tank and the minimum mean number of bacteria (mean 4.0 CFU/m³) was in February in the site from 100 m, distant from the plant, The majority of the air of the station was negative bacteria, and the proportion of *Enterobacteriaceae* was (50.4%). harmful bacteria, such as (*Aeromonas sp.*, *Klebsiella pneumoniae sp.*, *Acinetobacter sp.*, *Pseudomonas sp.*, and others) can cause risk impact on the employees in plant.

Keyword: WWTP, bioaerosols, Microorganism, Pathogenic and non-pathogenic bacteria

1. Introduction

Sewage treatment plants have been shown to produce some hazardous air pollutants, such as bioaerosols, during handling sewage to protect the environment, and have thus become a growing source of public concern(1). Wastewater treatment plants (WWTP) have the potential to harm the environment in a variety of ways. These effects are dependent on the size, technology, and treatment methods of the wastewater treatment plant. Viruses, bacteria, fungi, protozoa, and helminthes are among the pathogens found in wastewater, which originate from human activities in homes, businesses, and other institutions. During the treatment process at a WWTP, these microorganisms can easily become airborne (2). The formation of biological aerosols containing numerous microorganisms is one of the most serious threats (3). Living and non-living microorganism cells make up bioaerosols(4).

A primary source of bioaerosols released in the outdoor environment is agitation of open water, such as in WWTP aeration tanks(5). Workers at sewage treatment plants are frequently being subjected to biological agents, which can lead to diseases like alveolitis (6), infections of the gastrointestinal tract, rhinitis, pharyngitis, and conjunctivitis (7).The majority of bioaerosol particles emitted by sewage treatment plants are less than 4.7 mm in diameter, making them repairable. The particles can travel several kilometers on the wind, posing a threat to workers as well as local residents (8). These airborne bacteria contained some opportunistic pathogenic species. From the air emissions of WWTPs, *Acinetobacter sp.*, *Pseudomonas sp.*, *Aeromonas sp.*, *Klebsiella pneumoniae sp.*, and *Escherichia coli* were isolated. Additionally, among WWTP employees, an

increased prevalence of respiratory and intestinal diseases caused by airborne bacteria was reported (9). The aim of this study was conducted to (1) detect bacterial air pollution in wastewater treatment plants, (2) identify bacterial types in the air of the study area.

2. Materials and Methods

2.1. Study site and Sampling methods

The study took place at Hilla City's Al-Muamira wastewater treatment plant (Babil province, Iraq). From November 2021 to February 2022, bioaerosol air samples (Bacteria) were collected from five points, raw sewage entrance (point1), aeration tank (point2), Drying basins (point3), and treated sewage outflow (point 4) at distances of 100 meters from the plant (point 5).

Air samples were taken using open Petri dishes that contained (specific media to isolate bacteria) and the Petri was positioned for five minutes at a height of around 1.5 m and in the wind direction. Every month, three samples were taken from each point, and the sampling period was set between (9:00 and 11:00 morning). The Petri dishes were transported to the laboratory after sampling. Bacterial samples were grown in an incubator at 37 degrees Celsius. Bacterial colony development was observed within 2 days, and the number of colonies was calculated. A colony-forming unit, or CFU, served as a representation of the results.

2.2. Diagnosis of bacteria

1.VITEK® 2 Diagnosis System

Using VITEK® 2 Compact to identify all cultivated airborne microorganisms. Following 18 to 24 hours of cultivation on blood or McConkey agar, a few colonies of a pure culture were harvested using a

sterile microloop. A bacterial culture was calibrated using a VITEK® 2 DensiChek. If the gram stain was negative, the GN card was taped and placed inside the instrument. If the gram stain was positive, the GP card was taped and placed within the apparatus. Every 15 minutes, colorimetric measurements were carried out automatically when each card was taken out of the incubator. The outcomes were read after an incubation period of 10 to 18 hours(10).

2. Bacterial DNA Extraction

Identification of bacterial species by DNA extraction according to the manufacturer’s instructions (Bacteril Genomic DNA Extraction kit, Addprep, Korea) and this DNA was used as the template for PCR amplification . Universal primers 27F(5'AGA GTT TGA TCC TGG CTC -3') ,1492R (5'GGT TAC CTT GTT ACG ACT T-3') (11), were used to amplify the eubacteria 16S rDNA segments .

3. Results and Discussions

3.1. The mean number of Bacterial Colonies

The research was done during the cold months. The temperature of air between (10-16 °C) and relative humidity between (40-50 %). The results of the statistical analysis in Table 1 demonstrated that there were significant differences (P-value < 0.001) between all sites as well as was between months. The highest mean number of bacterial colonies (150.0 CFU/m3) in November had been seen in the aeration tank , followed by drying basins , where the highest average (50.0 CFU/m3) was in December, followed the Raw sewage entrance , where the highest average (30.0 CFU/m3) was recorded in November , followed treated sewage outflow, where it reached the maximum mean number of colonies (25.0 CFU/m3) was in the December ,followed by from 100 distant of plant where the highest average (9.3 CFU/m3) was in December. In general, the minimum mean of bacterial colonies (4.0 CFU/m3) was in February in the site from 100 m, distant from the plant, and the maximum mean of bacterial colonies (150.0 CFU/m3) in November had been seen in the aeration tank.

Table 1: Means and comparison average of total bacteria (based on CFU/m3) across several sampling points and months (P value).						
Total Bacteria 1000 CFU /m3						
Point Months	Point 1 Mean	Point 2 Mean	Point 3 Mean	Point 4 Mean	Point 5 Mean	P value
November	30.0	150.0	46.0	20.7	8.0	0.000*
December	29.0	140.0	50.0	25.0	9.3	0.000*
January	16.0	100.0	20.0	11.0	5.0	0.000*
February	20.0	95.0	27.0	7.0	4.0	0.000*
P value	0.000**	0.000**	0.000**	0.000**	0.000**	
Total	23.75	121.25	35.75	15.92	6.575	

** means high significance differences (P-value < 0.001)

According to findings in Table 1 the most contamination sites was aeration tank. The surface

aeration system at the sewage treatment facility that was examined causes a significant disruption in the aeration tank, increasing the amount of bioaerosols in the air by bursting water bubbles that release microorganisms. The study conducted to Fathi et al., the aeration tank ' had the highest average airborne bacterial concentration. According to recent study by Ruiz-Gil et al. ,Maximum concentrations of were seen in bioaerosols during the aeration stage (12). The number of colonies in the cold months was few, where the maximum was recorded during November, due to the low temperature during the cold months, and the temperature is one of the necessary factors metabolic and reproduction rates of bacteria (13,14).

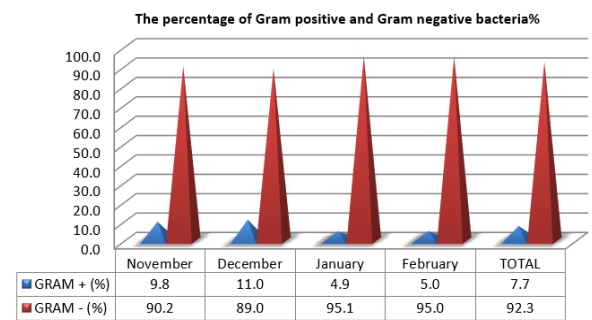


Figure 1: The proportion of Gram positive and Gram negative in cold months

Species community

As seen in Figure 1, approximately (92.3 %) of the bacteria in the WWTP are Gram-negative bacteria, whereas the remaining (7.7%) are Gram-positive bacteria. Gram-negative bacteria may be more common than positive bacteria because of their capacity to endure harsh conditions (15). As present in Figure 2, February had the highest percentage (64.3 %) of *Enterobacteriaceae*, followed by January with a percentage of (54.6%), November with a percentage of (46.1%), and December with a percentage of (36.6%). and the average percentage (52.4%). several *Enterobacteriaceae* species has been isolated such as (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella pneumonia ssp ozaenae*, *Raoultella planticola* , *Serratia fonticola* and *Pantoea* . Pathogenic bacteria such as (such as *Aeromonas sp.*, *Klebsiella pneumoniae sp.*, *Acinetobacter sp.*, *Pseudomonas*, and others). The presence of these bacteria in very small quantities in the air is harmful to workers and employees who suffer from immunodeficiency may be exposed to it. Table 2 lists the species of bacteria that were identified during this research study.

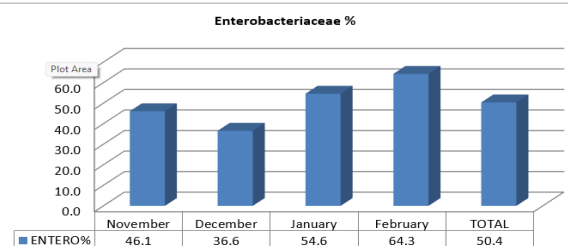


Figure 2: The proportion of Enterobacteriaceae found in the air at the WWTPs.

Table 2: lists the species of bacteria that were identified during this research study

Gram- positive bacteria	Gram-negative bacteria non Enterobacteriaceae	Gram negative Enterobacteriaceae
Staphylococcus vitulinus (2,4,5) Bacillus badius (4) bacillus cereus (3) Kocuria kristinae (3,4,5) Kocuria rhizophila (4)	Chromobacterium violaceum (1) Sphingomonas paucimobilis (1,2,3,4) Sphingomonas thalpophium (3) Acinetobacter lwoffii (1,2,3) Acinetobacter calcoaceticus (1) Acinetobacter ursingii (5) Aeromonas hydrophila \ punctata(caviae) (2,3,4) Aeromonas sobria (2,3,5) Aeromonas salmonicida (2,4,5) Aeromonas veronii(4) Pseudomonas fluorescens (3) Pseudomonas aeruginosa (2) Pseudomonas alcaligenes (4) Pseudomans luteola (3) Leclercia adecarboxylata (1,3,4)	Escherichia coli (1,2,3,4) Pantoea spp (1,2) Klebsiella pneumoniae ssp pneumonia (1,2,3,4) Klebsiella pneumoniae ssp ozaenae (1) Citrobacter freundii (3,4) Serratia fonticola(1,2) Raoultella planticola (2,3)
*The locations of isolation are listed in parenthesis		

4. Conclusion

At WWTPs, aeration tank is the main source of bioaerosol emissions. Our results indicated bacterial growth decreases in the colder months and the main environment factor contributed on bacteria growth is temperature. Exposing workers to small amounts of bioaerosols can cause health problems.

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